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L4: Entry 1 of 58

File: PGPB

Jan 20, 2005

DOCUMENT-IDENTIFIER: US 20050015830 A1

TITLE: Method of protein production in plants

Brief Description of Drawings Paragraph:

[0067] FIG. 6 depicts the cloning scheme of different GFP-PME fusions in a plant expression cassette for transient expression experiments.

Detail Description Paragraph:

[0101] The nucleic acid having the coding region coding for the protein of interest with a secretory signal peptide and a cell wall binding domain can be designed in many different ways. Usually, the signal peptide is localized at the N-terminal end of the fusion protein. The cell wall binding domain(s) can link the signal peptide with the protein of interest or can be located at the C-terminal end of said fusion protein, or two cell wall binding domains may surround the protein of interest. In one embodiment of this invention, the whole tobacco pectin methylesterase (PME cDNA, see FIG. 2; PME protein precursors, see FIG. 3) gene was fused with a green fluorescent protein (GFP) gene as a protein of interest. Pectin methylesterase (PME) (Gaffe et al., 1997, Plant Physiol., 114, 1547-1556; Dorokhov et al., 1999, FEBS Lett., 461, 223-228) is a secretory protein, which can be detected in the endoplasmatic reticulum (ER) and the cell wall. The members of the PME multigene family that undergo post-translational processing (Gaffe et al., 1997, Plant Physiol., 114, 1547-1556), are involved in cell wall turnover and appear to have a role in plant growth and development (Wen et al., 1999, Plant Cell, 11, 1129-1140). PME is known to be a ubiquitous enzyme in the plant kingdom and regulates the cell wall degradation by catalyzing the demethoxylation of pectins. Mature 33 kDa PME is localized in the cell wall, while PME precursor is synthesized as 70 kDa polyprotein. It is obvious that PME processing and trafficking to cell wall is accompanied by the removal of the N-terminal leader sequence, whereas anchoring of PME in the cell wall requires the specific pectin binding domain.

Detail Description Paragraph:

[0164] Construction of Vectors Carrying Different Types of GFP Fusions with the Tobacco Pectin Methylesterase (PME) Gene for Transient Expression and Stable Nuclear Transformation of Plants

Detail Description Paragraph:

[0201] The binary vector-based translational fusion of GFP with the tobacco cell wall protein TLPR (Domingo et al., 1999, Plant J., 20, 563-570; Accession number Y19032) was performed in the same way as described for PME-GFP fusion, except for different primers.

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L1: Entry 4 of 8

File: USPT

Aug 7, 2001

US-PAT-NO: 6271033

DOCUMENT-IDENTIFIER: US 6271033 B1

TITLE: Method for modifying production of fruit ripening enzyme

DATE-ISSUED: August 7, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bridges; Ian G.	Slater	IA		
Grierson; Donald	Loughbrough			GB
Schuch; Wolfgang W.	Crowthorne			GB

US-CL-CURRENT: 435/468

## CLAIMS:

We claim:

1. A process for modifying the production of a target gene product in a plant cell which comprises transforming the plant cell with a construct comprising a recombinant DNA sequence coding for only part of the target gene product wherein said target gene product is a fruit ripening enzyme.
2. Process as claimed in claim 1 in which the fruit-ripening enzyme is polygalacturonase.
3. The process of claim 1 wherein the gene product is polygalacturonase or pectinesterase, said recombinant DNA sequence being shorter than the sequence encoding polygalacturonase or pectinesterase but sufficient to inhibit the expression of said polygalacturonase or pectinesterase.
4. The process of claim 3 wherein the plant cells are tomato plant cells.
5. The process of claim 1 wherein the enzyme is pectinesterase, galactosidase, glucanase, xylanase or cellulase.
6. The process of claim 5 wherein the recombinant DNA sequence comprises at least 50 bases of the gene encoding said enzyme.
7. The process of claim 6 wherein the DNA sequence comprises up to 1000 bases of said gene.
8. The process of claim 1 wherein the plant cells are tomato cells and the construct is selected from the group consisting of pJR36S, pJR56S, pJR76S, pJR26S, pJR46S, pJR66S, pJR86S, pJR101S, pJR111S, pJR102S and pJR112S.

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☐ 1. Document ID: US 6727406 B2

L1: Entry 1 of 8

File: USPT

Apr 27, 2004

US-PAT-NO: 6727406

DOCUMENT-IDENTIFIER: US 6727406 B2

TITLE: Purified proteins, recombinant DNA sequences and processes for controlling the ripening of coffee plants

DATE-ISSUED: April 27, 2004

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stiles; John I.	Kaneohe	HI		
Moisyadi; Istefo	Honolulu	HI		
Neupane; Kabi Raj	Honolulu	HI		

US-CL-CURRENT: [800/283](#); [435/183](#), [435/189](#), [435/320.1](#), [435/419](#), [435/468](#), [536/23.2](#), [536/23.6](#), [536/24.1](#), [536/24.5](#), [800/278](#), [800/286](#), [800/287](#), [800/295](#), [800/298](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw Ds
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☐ 2. Document ID: US 6355862 B1

L1: Entry 2 of 8

File: USPT

Mar 12, 2002

US-PAT-NO: 6355862

DOCUMENT-IDENTIFIER: US 6355862 B1

**\*\* See image for Certificate of Correction \*\***

TITLE: Fruit quality by inhibiting production of lipoxygenase in fruits

DATE-ISSUED: March 12, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Handa; Avtar K.	West Lafayette	IN		
Kausch; Kurt D.	Chicago	IL		

US-CL-CURRENT: [800/290](#); [435/193](#), [435/320.1](#), [435/411](#), [435/419](#), [435/468](#), [536/23.6](#), [800/278](#), [800/283](#), [800/284](#), [800/286](#), [800/317.4](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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☐ 3. Document ID: US 6348641 B1

L1: Entry 3 of 8

File: USPT

Feb 19, 2002

US-PAT-NO: 6348641

DOCUMENT-IDENTIFIER: US 6348641 B1

TITLE: Purified proteins, recombinant DNA sequences and processes for producing  
caffeine-free beverages

DATE-ISSUED: February 19, 2002

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Stiles; John I.	Kaneohe	HI		
Moisyadi; Istefo	Honolulu	HI		
Neupane; Kabi Raj	Honolulu	HI		

US-CL-CURRENT: 800/285; 435/193, 435/320.1, 435/419, 435/468, 536/23.6, 800/278,  
800/286

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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☐ 4. Document ID: US 6271033 B1

L1: Entry 4 of 8

File: USPT

Aug 7, 2001

US-PAT-NO: 6271033

DOCUMENT-IDENTIFIER: US 6271033 B1

TITLE: Method for modifying production of fruit ripening enzyme

DATE-ISSUED: August 7, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bridges; Ian G.	Slater	IA		
Grierson; Donald	Loughbrough			GB
Schuch; Wolfgang W.	Crowthorne			GB

US-CL-CURRENT: 435/468

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw. D.
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☐ 5. Document ID: US 5744098 A

L1: Entry 5 of 8

File: USPT

Apr 28, 1998

US-PAT-NO: 5744098  
DOCUMENT-IDENTIFIER: US 5744098 A

TITLE: Device for the automatic examination of blood samples

DATE-ISSUED: April 28, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kratzer; Michael	Munich			DE
von der Goltz; Volker Freiherr	Seon			DE

US-CL-CURRENT: 422/73; 422/101, 422/102, 73/64.41

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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☐ 6. Document ID: US 5460779 A

L1: Entry 6 of 8

File: USPT

Oct 24, 1995

US-PAT-NO: 5460779  
DOCUMENT-IDENTIFIER: US 5460779 A

TITLE: Device for the automatic examination of blood samples

DATE-ISSUED: October 24, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kratzer; Michael	Munich			DE
von der Goltz; Volker F.	Seon			DE

US-CL-CURRENT: 422/73; 422/58, 422/63, 422/66, 422/69, 436/48, 436/69, 73/64.41

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KWIC	Draw D
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☐ 7. Document ID: US 5413937 A

L1: Entry 7 of 8

File: USPT

May 9, 1995

US-PAT-NO: 5413937  
DOCUMENT-IDENTIFIER: US 5413937 A

TITLE: DNA constructs containing segments from tomato polygalacturonase and pectin esterase genes

DATE-ISSUED: May 9, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Bridges; Ian G.	Slater	IA	
Grierson; Donald	Loughbrough		GB2
Schuch; Wolfgang	Crowthorne		GB2

US-CL-CURRENT: 435/320.1

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KMC	Draw De
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☐ 8. Document ID: US 5296376 A

L1: Entry 8 of 8

File: USPT

Mar 22, 1994

US-PAT-NO: 5296376DOCUMENT-IDENTIFIER: US 5296376 A

TITLE: DNA, constructs, cells and plants derived therefrom

DATE-ISSUED: March 22, 1994

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Bridges; Ian G.	Slater	IA		
Grierson; Donald	Loughbrough			GB2
Schuch; Wolfgang W.	Crowthorne			GB2

US-CL-CURRENT: 435/320.1; 800/317.4

Full	Title	Citation	Front	Review	Classification	Date	Reference			Claims	KMC	Draw De
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L4: Entry 31 of 58

File: USPT

Jul 4, 2000

US-PAT-NO: 6083540

DOCUMENT-IDENTIFIER: US 6083540 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Process for stabilizing proteins in an acidic environment with a high-ester pectin

DATE-ISSUED: July 4, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christensen; Tove Martel Ida Elsa	Allerod			DK
Kreiberg; Jette Dina	Roskilde			DK
Thorsoe; Hanne	Aarhus C			DK
Buchholt; Hans Christian	Brabrand			DK
Rasmussen; Preben	Lyngby			DK
Nielsen; John	Copenhagen			DK

US-CL-CURRENT: 426/50; 426/52

CLAIMS:

What is claimed is:

1. A method of block-wise enzymatically de-esterifying a pectin comprising the step of:

treating the pectin with a recombinant enzyme comprising any amino acid selected from the group consisting of: SEQ ID NO:1, SEQ ID NO:2, and a variant, derivative or homologue thereof.

2. The method of claim 1, wherein the block-wise enzymatically degraded pectin is prepared by treating a pectin with a recombinant enzyme that is obtainable by expression of the PME coding sequence contained in NCIMB 40749 or NCIMB 40750, or a variant, derivative or homologue thereof.

3. The method of claim 1, wherein the block-wise enzymatically de-esterified pectin is prepared by treating the pectin with the recombinant pectin methyl esterase in the presence of sodium ions.

4. The method of claim 3, wherein the block-wise enzymatically de-esterified pectin is prepared by treating the pectin with the recombinant pectin methyl esterase in the presence of a salt selected from the group consisting of NaCl, NaNO<sub>3</sub> and Na<sub>2</sub>SO<sub>4</sub>.

5. The method of claim 1, wherein the block-wise enzymatically degraded pectin

is prepared by treating a pectin with a recombinant enzyme that is obtainable by expression of the PME coding sequence contained in NCIMB 40749 or NCIMB 40750.

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File: USPT

Sep 30, 2003

US-PAT-NO: 6627429

DOCUMENT-IDENTIFIER: US 6627429 B1

TITLE: Process for enzymatically modifying pectin

DATE-ISSUED: September 30, 2003

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Christensen; Tove Martel Ida Else	Allerod			DK
Pedersen; Anette Amstrup	Soborg			DK
Brunstedt; Janne	Roskilde			DK
Mikkelsen; Jorn Dalgaard	Hvidovre			DK

US-CL-CURRENT: [435/275](#); [435/101](#), [435/183](#), [435/197](#), [435/243](#), [435/252.1](#), [435/252.3](#), [435/254.1](#), [435/41](#), [435/72](#)

## CLAIMS:

What is claimed is:

1. A process for treating a pectin with pectin methyl esterase (PME) to produce a PME-treated high ester pectin, comprising the step of contacting a pectin with an Erwinia PME, having a pH optimum with lime pectin of about pH 7.0, that is capable of block-wise de-esterification of the pectin, to produce a PME-treated high ester pectin that contains from about 70% to about 80% ester groups.
2. The process according to claim 1 wherein the PME has a molecular weight of about 36,000 D and/or a pI of about >9 and/or a temperature optimum with lime pectin of about 48.degree. C.
3. The process according to claim 1 wherein the PME comprises the amino acid sequence shown as SEQ.I.D. No.2, or an amino acid sequence having PME activity with at least 75% homology to SEQ ID No:2.
4. The process according to claim 1 wherein the PME has the amino acid sequence shown as SEQ.I.D. No.2, or an amino acid sequence having PME activity with at least 75% homology to SEQ ID No:2.
5. The process according to claim 1 wherein the PME has the amino acid sequence shown as SEQ.I.D. No.2.
6. The process according to claim 1 wherein the PME has been expressed by a nucleotide sequence comprising the nucleotide sequence shown as SEQ.I.D. No.1, or a nucleotide sequence with at least 75% homology to SEQ ID No:1, or combinations thereof.

7. The process according to claim 1 wherein the PME has been expressed by a nucleotide sequence having the nucleotide sequence shown as SEQ.I.D. No.1, or a nucleotide sequence with at least 75% homology to SEQ ID No:1.
8. The process according to claim 1 wherein the PME has been expressed by a nucleotide sequence having the nucleotide sequence shown as SEQ.I.D. No.1.
9. The process according to claim 1 wherein the PME has been prepared by use of known recombinant DNA techniques.
10. The process according to claim 1 wherein the pectin is in contact with the PME in the presence of sodium ions.
11. The process according to claim 10 wherein the sodium ions are derived from NaCl, NaNO.sub.3, or Na.sub.2 SO.sub.4 or combinations thereof.
12. The process according to claim 1 wherein the process includes the further step of isolating the PME-treated pectin from remaining active PME.
13. The process according to claim 12 wherein the PME treated pectin contains from about 72% to about 80% ester groups.
14. The process according to claim 12 wherein the PME treated pectin contains from about 74% to about 80% ester groups.
15. The process according to claim 12 wherein the PME treated pectin contains from about 76% to about 80% ester groups.
16. The process according to claim 12 wherein the PME treated pectin contains from about 77% to about 80% ester groups.
17. The process according to claim 1 wherein the process includes the further step of adding the PME-treated pectin to a medium that is suitable for human or animal consumption.
18. The process according to claim 17 wherein the medium is an aqueous solution.
19. The process according to claim 18 wherein the aqueous solution is a beverage.
20. The process according to claim 17 wherein the medium is an acidic environment.
21. The process according to claim 20, wherein the acidic environment has a pH of from about 3.5 to about 5.5.
22. The process according to claim 21 wherein the acidic environment has a pH of about 4.
23. The process according to claim 19, wherein the beverage is a acidified milk drink.
24. The process according to claim 17 herein the medium of comprises a

protein.

25. The process according to claim 22 wherein the protein is derived from or is obtainable from or is in a dairy product.

26. The process according to claim 22 wherein the protein is derived from or is obtainable from or is in a plant product.

27. The process according to claim 20, wherein the acidic environment has a pH of from 4 to about 5.5.

28. A method for reducing the number of ester groups in a pectin in a block-wise manner, comprising the step of contacting a pectin with a pectin methyl esterase (PME) to produce a PME-treated pectin that contains about 70% to about 80% ester groups and which PME comprises the amino acid sequence shown as SEQ.I.D. No.2 or an amino acid sequence with at least 75% homology to SEQ ID No:2, wherein said PME is not a plant PME and is capable of block-wise de-esterification of the pectin.

29. A method for de-esterifying two or more adjacent galacturonic acid residues of a pectin on a pectin chain, comprising the step of contacting a pectin with a pectin methyl esterase (PME) to produce a PME-treated pectin that contains about 70% to about 80% ester groups and which PME comprises the amino acid sequence shown as SEQ.I.D. No.2 or an amino acid sequence with at least 75% homology to SEQ ID No:2, wherein said PME is not a plant PME and is capable of block-wise de-esterification of the pectin.

30. The process according to claim 28 or 29 wherein the PME is obtained from a micro-organism.

31. The process according to claim 30 wherein the PME is obtained from a bacterium.

32. The process according to claim 17, wherein the medium is enriched with a protein.

33. The process according to claim 32 wherein the protein is derived from or is obtainable from or is in a dairy product.

34. The process according to claim 32 wherein the protein is derived from or is obtainable from or is in a plant product.

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